

Memorandum

HSM-90002

(Numbered after original issuance)

To : Dr. Robert I. Krieger
Chief/Supervising Toxicologist

Date: March 23, 1990

Place: Sacramento

324-6149

From : Department of Food and Agriculture - Clifford R. Smith
Senior Environmental Hazards Scientist

Subject: Discussion/Decision Document on Whole Leaf Dislodgeable Foliar Residue
(WLD FR) Sampling

As you may recall, we collected a series of whole grape leaves last year in order to evaluate three methods of analyzing whole leaves for dislodgeable residue and compare them with discs analyzed by the traditional method. The goal at the time was to provide the laboratory with an adequate data base on which to recommend a whole leaf dislodgeable method to private (certified) laboratories working with growers seeking to abbreviate methomyl reentry intervals. A set of samples was collected from one field only; the results are attached, including discussion by Mercy DelValle. Also attached are solicited comments from Dr. Dong.

Ideally, we would have a broader data set before drawing any conclusions, but I believe it is sufficient to rethink our approach to WLD FR sampling. Following are some statements for further discussion (maybe even agreement?) resulting from this and other work we've done recently.

1. Sample size, in terms of numbers of leaves is not a critical issue in the range of sampling (10 to 40). Future decisions on sample-size can be based on statistical representation and sample handling logistics within the range of 20 to 40 leaves.
2. Of the three methods used (rolling in a jar, shaking in a jar, shaking in a bag), shaking in a jar was the most effective method of dislodging methomyl. It was also the most easily standardized method, using commonly available apparatus. All methods removed more than the DFR by leaf disc. Only the comparatively least effective WLD FR method, manual shaking of a bag, could be considered similar to discs.
3. It is apparent that none of the methods tried can be used for the original regulatory purpose without either being attenuated by inefficiency or having an equivalence factor applied. Neither is an attractive prospect, even putting aside questions of scientific validity. All of our reentry intervals, including methomyl, are unavoidably based on leaf discs as the index of exposure. We should recommend that only leaf disc DFRs be applied to the reentry interval for the purpose of abbreviating a reentry interval. If whole leaf samples are received by a laboratory, the lab should have a punch to sample the leaves. The nature and conveyance of this recommendation is the responsibility of Chemistry Laboratory Services.

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4. It is also apparent that pursuing and developing the best possible whole leaf method could contribute to our understanding of the dynamics of foliar pesticide residues. We should continue to evaluate WLDFR methods and develop the best possible procedure for experimental purposes. Extraction in a jar mounted on a shaker table, should be the basis for future work, with other analytical parameters to be optimized.
5. This data set is further documentation of the potential for sampler bias to influence results, **significantly**. (Replicate B was collected by one sampler, Rep A by another, for Rep C it is not clear who collected which samples. A visual comparison of Reps A and B leaves no doubt of the significance.) Principle investigators need to clearly specify the sampling population and ensure a common understanding of same within the sampling team.

The real cause for the difference between WLDFR and leaf disc DFRs, especially in grapes, is probably systematic sampling bias (from leaf punches). We have little data to support this, but focusing our WLDFR efforts in this area will be more productive than developing a method that reproduces the current DFR data base.

cc: John Ross
Jim Sanborn
Michael Dong
Sue Edmiston
Linda O'Connell
Stan Bissell
Terry Jackson, Chemistry Laboratory Services
Mercy DelValle, Chemistry Laboratory Services
Dave Conrad, Chemistry Laboratory Services
Chuck Andrews, Pesticide Enforcement

FYI - Results of last year's whole leaf dislodgeable extraction methods evaluation.

Method of Dislodging

Method 1: Place leaves in a one-gallon plastic jar together with the required amount of water and aerosol OT-75 solution and place jars on a roller for 30 minutes.

10 leaves - 400 ml H₂O and 1/2 dropper-full of two percent OT-75

20,30,40 leaves - 800 ml H₂O and one dropper-full of two percent OT-75

Method 2: Place leaves in jar with required amounts of H₂O and OT-75 solution as above and place jars on a shaker table for 30 minutes.

Method 3: Add the H₂O and OT-75 solution in the plastic bag containing the sample. Seal the bag and shake by hand for two minutes.

10 and 20 leaves - 200 ml H₂O and four drops of two percent OT-75 solution

30 and 40 leaves - 400 ml H₂O and 1/2 dropper-full of two percent OT-75 solution

Extraction for all samples:

Take 200 ml aliquot and extract with 3 X 50 ml CH₂Cl₂ . Evaporate and exchange into 5 ml methanol.

Conclusions

Method of Dislodging:

Method 1 (rolling in one-gallon jars) gave the highest values for 20 leaves but for 20 leaves and higher, Method 2 (shaking in one-gallon jars) gave the highest values. The reason for this might be that for 20 or more leaves, the sample is too crowded in the jar and rolling does not give enough agitation for water to get between the leaves. Method 3 and the punches gave lowest values in all cases.

Number of leaves per sample:

No definite conclusion can be made as to the optimum number of leaves per sample. Replicate samples have to be collected for one set of variables and analyzed again for the sample size that will give the most representative estimate and the most consistent results.

Ten leaves per sample using Method 1 seem to give representative values for a site, although it did not give the highest value for all sites.

Whole Leaves	Sampling Study Methomyl (ug/cm ²)	Mdel Valle 3/8/90
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	Method 1	Method 2	Method 3
10A	0.27+	0.19	0.14
10B	0.22+	0.16	0.06
10C	0.30+	0.23	0.25
20A	0.23	0.31+	0.11
20B	0.04	0.26+	0.04
20C	0.23	0.25+	0.20
30A	0.27	0.34+	0.14
30B	0.08	0.15+	0.03
30C	0.16	0.28+	0.14
40A	0.18	0.25+	0.18
40B	0.13	0.17+	0.11
40C	0.29	0.28+	0.14

+ - highest value for a row (same number of leaves, same site)

Punches (40 punches/sample)

A1 - 0.11 ug/cm ²	
A2 - 0.08	} Average - 0.09 ug/cm ²
A3 - 0.07	
B1 - 0.07	
B2 - 0.07) Average - 0.06 ug/cm ²
B3 - 0.05	
C1 - 0.18	
C2 - 0.08) Average - 0.13 ug/cm ²
C3 - 0.13	

Whole Leaves	Methomyl (ug/cm2)		
	Site A	Site B	Site
10 (M1)	0.27	0.22	0.30+
10 (M2)	0.19	0.16	0.23
10 (M3)	0.14	0.06	0.25
20 (M1)	0.23	0.04	0.23
20 (M2)	0.31	0.26+	0.25
20 (M3)	0.11	0.04	0.20
30 (M1)	0.27	0.08	0.16
30 (M2)	0.34+	0.15	0.28
30 (M3)	0.14	0.03	0.14
40 (M1)	0.18	0.13	0.29
40 (M2)	0.25	0.17	0.28
40 (M3)	0.18	0.11	0.14
+ - highest value for one site			
Punches (Average)	0.09	0.06	0.13

Memorandum

To John H. Ross, Senior Toxicologist
Worker Health and Safety Branch

Date March 21, 1990

Place Sacramento

Phone 445-8474

From Department of Food and Agriculture - Michael H. Dong, Staff Toxicologist
Worker Health and Safety Branch

Subject *Comments* on Results of Last Year's Whole Leaf Dislodgeable Extraction Methods Evaluation (see attached)

Recently a summary of the results of the above methods evaluation was made available to me. With further elaboration from Cliff Smith on the methodologies involved, I have made the following observations/speculations.

1. Among the three whole leaf methods, the method of using hand shaking (ie, Method 3) gave the lowest residue values per cm² leaf surface. The reason for this might be that the agitation from shaking by hand for *only 2 minutes* was not enough or comparable.
2. The leaf punch method in effect gave the lowest values in all cases. This is expected in the sense that pesticide sprays tend to run and accumulate near the leaf margins (or some other particular locations) and hence might not be uniformly distributed over the entire surface (from which a small portion may be taken as the disc sample). In addition, the leaf punch and the whole leaf methods might not give comparable agitation (which is a function of force and time).
3. Site B tended to provide those whole leaf or punch samples with the *least* amount of residues present.
4. If all methods of dislodging are to be repeated for further exploration, I recommend that each be performed with an additional effort to investigate the effect of its agitation (on dislodging) *over time* (eg, 10, 20, 30, 60 min). (The analyses should be done *blinded*.)
5. Insofar as the magnitude of underestimation (from the leaf punch method) remains unknown and tends to be crop/variety- or time-specific, I propose that both the punch and (the best of the) whole leaf methods be used side-by-side for a while. There is the notion that a worker is more likely to come in contact with a whole leaf (WL) indiscriminately than with the *specific* part from which the leaf punch (LP) sample is made. For this reason, it can be argued that *in the future as well as in the long run*, DFR_[WL] will serve as a better (or a more accurate) dermal exposure or reentry index than DFR_[LP].

cc: Bob Krieger
Cliff Smith
Dave Conrad

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